REMARKS

The Amendment

Entry of this amendment is respectfully requested. No new matter is added by the amendment as the amended claims find full support in the application as filed.

Claims 36-55 are in this application; Claims 15 and 16-35 having been canceled, and Claims 36-55 having been added by this amendment.

Claim 15 was rejected under 35 USC 112, ¶2. This rejection is believed moot in view of the cancellation of Claim 15. Claims 15-35 were rejected under 35 USC 103(a). These rejections are respectfully traversed as applied to the new claims.

The 35 USC 103(a) rejections

Claims 15-27 and 30-35 were rejected under 35 USC 103(a) as unpatentable over Cole et al., US Patent No. 4,110,165. The Examiner reasons that Cole et al. discloses fermentation of *Streptomyces clavuligerus* using a concentration of 0.2% KH_2PO_4 in the fermentation medium, and that this corresponds to about 0.11% assimilable phosphorus, within the claimed concentration range. He further reasons that while Cole et al. does not disclose specific examples of nitrogen concentration, it discloses that nitrogen concentrations of 0.1% to 10% may be used; and that fed batch and continuous processes and scale-up were well known and routine.

Claims 15-35 were rejected under 35 USC 103(a) as unpatentable over Cole et al. in view of Lawrence et al., GB Patent No. 1,571,888. The Examiner reasons that Cole et al. differs from Claims 28-29 in not disclosing the use of ammonium sulfate as the nitrogen source, but that Lawrence et al. teaches that inorganic salts including ammonium and sulfate ions can be used as nutrients in clavulanic acid fermentation.

Claims 15-35 were rejected under 35 USC 103(a) as unpatentable over Cole et al. in view of Lawrence et al. and further in view of Puentes et al., EP Published Application No. 0 182 522. The Examiner reasons that, with the exception of

S. clavuligerus, neither of Cole et al. nor Lawrence et al. disclose all the microorganisms related in Claim 32; but that Puentes et al. disclose that all of the microorganisms were known at the time of the invention to produce clavulanic acid.

Applicants respectfully disagree with respect to the new claims.

Cole et al. discloses KH_2PO_4 concentrations of 0.2% in Example 9, 0.1% in Example 12 (where the clavulanic acid yield was 200-300 µg/ml), and 0.1% in Example 13 (where the clavulanic acid yield was 300-500 µg/ml). No phosphorus was added during the fermentation. A review of the Examples in Cole et al. shows no relationship between the phosphate present in the starting fermentation medium and the yield of clavulanic acid achieved; but, while Cole et al. teaches that phosphate should be included in the fermentation medium, it does not state that it is essential nor teach any relationship between the phosphate added and the subsequent yield of clavulanic acid. Further, Cole et al. does not teach subsequent addition of phosphorus during the growth phase, as claimed in all claims.

Lawrence et al. teaches that the amount of clavulanic acid produced in fermentation of *S. clavuligerus* may be increased when the fermentation is carried out under strict pH control in the range pH 6.3-6.7; and Examples 1 through 3 disclose that the maximum clavulanic acid of the fermentation broth is 561 µg/ml, 754 µg/ml, and 550 µg/ml, respectively.

Puentes et al. teaches (page 3, last paragraph) that the fermentation and purification methods of available technology have produced impure clavulanic acid in relatively low yield; and discloses a method for the production of clavulanic acid where the source of assimilable carbon is added during the fermentation but the concentration is kept below 1%. In other words, Puentes et al. teaches control of catabolite repression. Example 1 discloses a starting concentration of KH_2PO_4 of 0.1%, but no phosphorus addition, and a clavulanic acid yield of 1403 µg/ml; while Examples 2 and 3 and the Comparative Examples show the effect of the addition of assimilable carbon (glycerol) during the fermentation. Example 4 shows a clavulanic acid yield of 1424

µg/ml. Puentes et al. also discloses no relationship between the starting phosphorus concentration and the clavulanic acid yield, nor does it show addition of phosphorus during fermentation.

The present application clearly discloses that the growth of a clavulanic acid-producing organism may include two phases, a growth phase during which biomass is produced, and a stationary phase during which growth does not occur. Secondary metabolites such as clavulanic acid are predominantly produced in the stationary phase.

The specification clearly states that the phosphorus concentration is preferably maintained below a limit of 0.15% during the growth phase, after which it may be allowed to decrease. The growth phase for a typical clavulanic acid fermentation lasting a total of 5-6 days may be completed by about 40 hours. Further limits on the phosphorus concentration are disclosed along with the associated benefits. The specification discloses that this concentration control produces improved yields of clavulanic acid.

In Example 1B of the application, 5 Kg (0.0083%) of NaH_2PO_4 is added to the starting medium and phosphate is added during the growth phase to maintain the phosphorus concentration below 0.15%. Example 1 discloses fermentation for a period of 96 hours, with a resultant clavulanic acid concentration of 3580 mg/l (3580 µg/ml) and during the growth phase of the fermentation a source of phosphorus was added in accordance with the table.

The following table compares the clavulanic acid yields of Cole et al., Lawrence et al., Puentes et al., and the present invention.

	Phosphorus source (phosphate) in the starting fermentation medium (%)	Phosphorus source (phosphate) added during fermentation (%)	Clavulanic acid yield in the fermentation medium (µg/ml)
Cole et al.	Ex. 9: 0.2 Ex. 12: 0.1 Ex. 13: 0.1	none	 Ex. 12: 200-300 Ex. 13: 300-500
Lawrence et al.			Ex. 1: 561 Ex. 2: 754 Ex. 3: 550
Puentes et al.	Ex. 1: 0.1 Ex. 4: 0.1		Ex. 1: 1403 Ex. 4: 1424
Present invention	Ex. 1: 0.0083	less than 0.15% (0.0015%-0.15%)	Ex. 1 3580

As can be clearly seen, the starting fermentation broth contains phosphorus in a lower concentration than the prior art. Additional phosphorus is added during the growth phase of the fermentation, but the amount is maintained below 0.15%. The source of phosphorus may be phosphate, e.g. sodium or potassium phosphate, disodium or dipotassium hydrogen phosphate, sodium or potassium dihydrogen phosphate, or mixtures thereof; with sodium dihydrogen phosphate being preferred.

This addition of phosphorus while maintaining the phosphorus concentration below 0.15% affords an unexpectedly higher yield of clavulanic acid, significantly greater than in the prior art, and neither taught nor suggested in the prior art.

The present application further teaches (at page 3) that the starting medium may contain assimilable carbon sources greater than 5%, and that further quantities of the carbon source may be added during the fermentation in accordance with usual fed batch principles; and that further quantities of the nitrogen source may be added during fermentation.

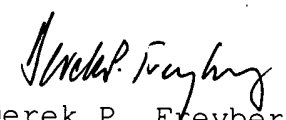
Applicants respectfully submit that the concentration of phosphorus and addition during the growth phase of fermentation,

and the other limitations in the dependent claims are neither disclosed nor suggested by Cole et al., alone or in combination with Lawrence et al., either alone or in view of Puentes et al.; and that the claims are therefore unobvious under 35 USC 103(a). Withdrawal of the rejections is therefore requested.

Conclusion

For the reasons given above, Applicants respectfully request that the amendment be entered, and that Claims 36-55 be allowed.

Respectfully submitted,



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